633

FEED YEAST FROM DAIRY BY-PRODUCTS[1]

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The possibility of producing feed yeast from dairy by-products and wastes was suggested from a previous study on the biochemical oxidation of dilute dairy waste. In that investigation *Saccharomyces fragilis* removed the lactose and casein, leaving in the effluent only a small amount of the original organic matter (10). As shown by Whittier (20) and Webb and Whittier (18), the use of whey in various fermentations is not novel, but its practicability depends upon economic considerations and the availability of suitable organisms. Various yeasts ferment lactose to alcohol (1, 11, 19), and details of such a process using *Torula cremoris* have been published (13). Good yields of whey yeast have been obtained with *S. fragilis* (2); in Germany, whey yeast plants operating in conjunction with dairies use *Torula* species (5).

Yeasts, that grow on dairy products are higher in protein and amino nitrogen than other yeasts; they also are high in ascorbic acid, thiamin and riboflavin (14). Riboflavin apparently is synthesized by yeasts that utilize lactose (12). Thus, the propagation of yeasts upon whey, skimmilk and dairy wastes may offer a means of obtaining an enriched feed supplement from soluble dairy products and at the same time reduce the pollution load.

PROCEDURES

Powdered skimmilk and powdered whey of the average composition shown in table 1 were dissolved in distilled water to the desired concentrations. All propagations were conducted at 30° C. Cultures used as inocula were cultivated for 65 hr. in Roux flasks containing 150 ml. of the indicated medium prior to use as seed. Filtered air was passed through the larger propagators at the rate of one volume of air per volume of fermenting solution per minute.

Determinations were made for lactose (16), protein (7), chemical oxygen demand as a measure of organic matter (9), dried centrifuged solids and direct yeast count.

Contamination was controlled by addition of 1 ml. of concentrated nitric acid for every 2 l. of solution in the fermentor when contamination was noticed. Such treatment, which does not seem to interfere with the growth of yeasts, is used for controlling infection at the Württemberg whey yeast plant (5).

skimmilk solutions were used as inocula. Twelve and a half ml. of a culture were used to seed 250 ml. sterile 0.1 per cent skimmilk contained in a 500-ml. gas washing bottle equipped with a fritted glass disc. After the solutions were aerated for 48 hr. at a rate of 125 ml. air per vessel per minute, determinations were made to obtain the data presented in table 2. These are the results obtained with a selected few of the 20 yeasts examined.

TABLE 1

Composition of powdered milk products used

	Skimmilk	Whey
Lactose (%)	50.5	76.5
Protein (%)	36.9	12.5
Ash (%)	8.1	6.0
Moisture (%)	4.5	4.9
Total solids (%)	95.5	95.0
Organic solids (%)	87.4	89.0

S. fragilis, NRRL 1109, gave the highest yield of solids. It removed large amounts of lactose and protein from the solution. After the solution was centrifuged, a supernatant solution low in organic matter, as measured in terms of chemical oxygen demand (C.O.D.), remained. *Candida lypolytica* and *T. utilis* utilized only small amounts of these solubles and the solids yields were low. The other organisms may find application, but *S. fragilis* was selected for further study and *T. cremoris* was used later for some comparative tests. The percentage of the original C.O.D. found in the solids (table 2) approximated the percentage of total solids recovered as centrifuged solids. It is interesting to note that *S.*

TABLE 2

Action of yeasts on 0.1% skimmilk solution after 48-hr. aeration[a]

Organism	NRRL strain	Lactose used	Total nitrogen removed	C.O.D. supernatant	C.O.D. solids	Solids recovered
		(%)	(%)	(%[b])	(%[b])	(%[c])
S. fragilis	1109	79	78	27	50	48
Z. lactis	1114	86	75	25	46	45
Z. casei	1564	85	71	26	34	39
T. cremoris	1131	59	57	34	46	43
C. lypolytica	1094	7	22	82	6	15
T. utilis	900	0	9	95	5	5

[a] Solution had 547 ppm. lactose, 370 ppm. protein and 1,117 ppm. C.O.D.
[b] Based on the original chemical oxygen demand of the skimmilk.
[c] Based on total solids originally in the skimmilk solution.

fragilis dissipated only about 23 per cent of the original C.O.D., possibly as CO_2.

Higher milk concentrations and ammonium sulfate additions. Addition of 11.25 ppm. of nitrogen to a 0.1 per cent skimmilk solution inoculated with *S. fragilis* resulted in good removal of organic matter (10). Increasing the milk solids as shown in table 3 required additional amounts of $(NH_4)_2SO_4$ for more complete utilization of the lactose and formation of removable solids. In the

24-hr. period, the number of yeast cells increased from 2.9, 7.3 and 14.5 million for the respective milk concentrations to the numbers given in the table. Although skimmilk contains nitrogen, it is apparent that a more readily available source is required for increased activity.

Batch production. Using the fermentor described by Humfield (4), 12-l. fermentations were made on a 2.5 per cent skimmilk solution and on a 2.5 per cent whey solution. $(NH_4)_2SO_4$ equal to 281 ppm. nitrogen was added. One l. of culture grown on the skimmilk solution was used for the former, and the same volume of culture grown on the whey solution was used for the latter. The yeast counts in the fermentor at the start were 9.7 million per milliliter for the milk and 3.9 million for the whey, reaching 525 million and 460 million at the end of the fermentations 13 and 16 hr. later, respectively. These values are comparable to those obtained with *S. cerevisiae* on grain wort (15). Sugar was utilized as rapidly as it was by *T. utilis* on peanut protein waste water, when 700 ppm.

TABLE 3

Action of S. fragilis grown for 24 hr. on skimmilk of different concentrations with added nitrogen
(The % values are based on the amount of that constituent present at the start)

Skim-milk solids	Nitrogen added as $(NH_4)_2SO_4$	Lactose used	Total nitrogen removed	Solids recovered	C.O.D. super-natant	Cells/ ml.	Increase in yeast
(g./l.)	*(ppm.)*	*(%)*	*(%)*	*(%)*	*(%)*	*(millions)*	*(fold)*
10	0.	30	26	19	50	49	17
10	11.3	49	37	24	26	62	21
10	113.	98	88	50	11	123	42
25	0.	39	49	13	49	100	13
25	11.3	46	60	33	32	122	16
25	282.	98	88	42	13	169	22
50	0.	49	24	11	56	194	12
50	11.3	63	48	15	51	250	16
50	563.	99	83	46	9	290	20

sugar were consumed in 5 hr. (6). The solids, recovered with a Sharples centrifuge, showed a yield of 46 per cent for skimmilk and 20 per cent for whey. Apparently, some of the protein in the skimmilk was precipitated at the acidity of the fermentation liquor (about pH 4).

Continuous propagation on skimmilk and whey. A 2-l. fermentor (3) that had been employed successfully in yeast production studies on fruit juices (17) was used in the following series of experiments. The starter was prepared in the fermentor, by aerating gently for 40 hr. the culture contents of one Roux flask and the indicated quantities of media. The feed to the fermentor was added from a calibrated funnel at such a rate as to keep the sugar in the fermenting solution below 10 mg. per milliliter. Antifoam (corn oil and lard) was added when necessary. The pH was maintained between 4 and 5 by the addition of NH_4OH.

Figure 1 shows the course of a fermentation in a solution containing 50 g. of skimmilk and 2.65 g. of $(NH_4)_2SO_4$ per liter (530 ppm. nitrogen). The starting volume was 450 ml. containing 130 million yeast cells per milliliter, or a total

of 58×10^9. At the end of the fermentation, 16 hr. later, the direct cell count was 514 million per milliliter, which for the 2-l. volume was a total of $1,028 \times 10^9$, a 17-fold increase. The pH gradually dropped to 4.2 at the completion of the run. The yield of dried recovered solids, when corrected for absorbed antifoam, was 45 per cent, based on the total milk solids. A Kjeldahl determination showed a protein content of 67 per cent, indicating a portion probably was precipitated casein. When the centrifuged solids were treated with NaOH to phenolphthalein pink, about one-half went into solution, indicating precipitated protein.

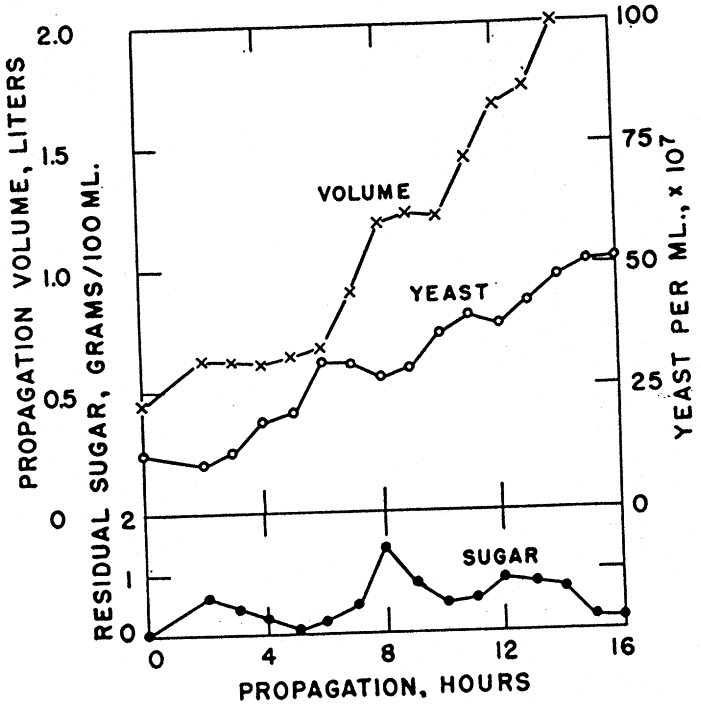

FIG. 1. Continuous propagation of *S. fragilis* on skimmilk. Feed contained 50 g. solids plus 2.65 g. $(NH_4)_2SO_4$ per liter.

A run made on a solution containing 50 g. of whey and 5.3 g. of $(NH_4)_2SO_4$ per liter (1,060 ppm. nitrogen) proceeded readily. In this case, the starter had a volume of 750 ml. and a yeast count of 500 million per ml. In 13 hr. when the run was completed, the yeast count was 633 million per ml. The total number of yeast cells had increased to $1,265 \times 10^9$ from the initial 381×10^9 cells. The dried solids showed a corrected recovery of 24 per cent, calculated to the original solids present. The protein content was 57 per cent, and treatment with NaOH showed the absence of soluble protein.

Foaming. Foaming was a major difficulty and necessitated constant surveillance during a propagation. Reference has been made to this same difficulty in the German whey plants (5). The low yields obtained in the present trials may have been caused by the means taken to control foaming. At times, the rates of air flow and agitation were markedly reduced, and the odor of alcohol was dis-

tinct. The antifoam agents used were not satisfactory. The silicones worked well with a 0.1 per cent skimmilk solution but not in higher solids concentration. Combinations of silicone and petrolatum proved ineffective when used in small amounts in the batch fermentations. Mixtures of corn oil and lard were not successful in the continuous fermentations because of the large amounts required. The removal of the foaming properties by coagulation was unsuccessful in the the following experiments.

Continuous propagation on clarified whey. Whey was clarified by running steam into a 20-l. bottle charged with 1,000 g. of whey and 10 l. of water. Steaming was continued for 20 min. after the temperature reached 99 to 100° C. After cooling and adjusting the whey solution to the 20-l. volume, the precipitated proteins were removed by filtering through paper pulp. For each 40 g. of lactose

TABLE 4

Propagation of S. fragilis on clarified whey

Hour	Yeast count $\times 10^7$/ml.	Lactose	Propagation volume
		(*g./100 ml.*)	(*ml.*)
0	42	3.63	200
1	47	1.99	240
2	44	2.60	330
4	45	2.16	380
5	56	1.56	490
6	47	1.40	580
7	48	1.22	645
9	44	.96	730
10	48	.68	810
11	49	.92	1045
12	52	.79	1195
13	48	.92	1305
14	54	.44	1505

in this solution, 1.2 g. $(NH_4)_2HPO_4$, 3.2 g. $(NH_4)_2SO_4$, and 1.55 ml. 28 per cent NH_3 (2) were added. The NH_3 was added after the solution was sterilized in liter flasks prior to use.

The cultured contents of three Roux flasks were aerated overnight. The yeasts were collected aseptically in centrifuge bottles, and the supernatant was replaced with 200 ml. of fresh medium. This served as the starting volume, which was increased by additions to a final volume of 1,500 ml. Tables 4 and 5 show data obtained on runs in which *S. fragilis* 1109 and *T. cremoris* 1131 were used.

S. fragilis increased about 10-fold and *T. cremoris* about 17-fold; however, the latter formed much smaller cells. Again, as shown earlier, *S. fragilis* utilized more sugar. Clarification of the whey did not seem to decrease its foaming properties. The rapid C.O.D. test showed that the yield calculated on the sugar utilized was 40 per cent, but based on the sugar available the yields were 35 per cent and 29 per cent for *S. fragilis* and *T. cremoris*, respectively.

The use of yeast for the disposal of dilute dairy waste by fermentation does not seem feasible. The considerable amount of oxygen-demanding substances that remains after the separation of solids still has a high percentage of the original pollution load. Further, fermentations of this type would require pure culture techniques with the necessity of pretreating the dilute wastes.

On the other hand, the propagation of desirable yeasts on skimmilk and whey offers a means of converting the soluble lactose and proteins to insoluble proteinaceous material. The water-soluble vitamins found in the by-products are not lost but are recovered in the enriched final product (8). Vigorous aeration poses a problem when milk products with their high foam-forming ability are used as nutrients for the yeast. Improved antifoam substances or fermentation equipment of the Waldhof type (5) will be required for efficient yeast production. Foaming is reduced only slightly by boiling whey and removing the coagulum.

TABLE 5

Propagation of T. cremoris on clarified whey

Hour	Yeast count $\times 10^7$/ml.	Lactose	Propagation volume
		(g./100 ml.)	*(ml.)*
0	54	3.40	200
2	56	2.41	385
3	86	1.90	410
5	83	1.22	410
6	84	1.80	620
8	68	1.46	800
9	93	1.28	800
10	110	1.26	875
11	96	1.64	1060
12	87	1.41	1270
13	88	1.39	1400
14	91	1.37	1500
15	122	1.19	1500

The selected yeast, *S. fragilis,* grew readily on unclarified and clarified whey and skimmilk. The recovered material was high in protein. It had only negligible amounts of B_{12}, 2–6γ per cent.[4] Yeast yields varying from 24 to 45 per cent were obtained from 5 per cent solutions of whey and skimmilk, with about 40 per cent conversion of the utilized sugar in a clarified whey. The possibility is suggested of producing an enriched protein product by such fermentations.

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[4] Vitamin B_{12} tests were made through the courtesy of J. C. Lewis, Biochemical Div. Western Regional Research Laboratory, Albany, Cal.

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